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15-Hydroxyprostaglandin Dehydrogenase Is Down-regulated in Colorectal Cancer*

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ABSTRACT

Prostaglandin E_2 (PGE_2) can stimulate tumor progression by modulating several proneoplastic pathways, including proliferation, angiogenesis, cell migration, invasion, and apoptosis. Although steady-state tissue levels of PGE_2 stem from relative rates of biosynthesis and breakdown, most reports examining PGE_2 have focused solely on the cyclooxygenase-dependent formation of

this bioactive lipid. Enzymatic degradation of PGE_2 involves the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). The present study examined a range of normal tissues in the human and mouse and found high levels of 15-PGDH in the large intestine. By contrast, the expression of *15-PGDH* is decreased in several colorectal carcinoma cell lines and in other human malignancies such as breast and lung carcinomas. Consistent with these findings, we observe diminished *15-Pgdh* expression in $Apc^{Min+/-}$ mouse adenomas. Enzymatic activity of 15-PGDH correlates with expression levels and the genetic disruption of *15-Pgdh* completely blocks production of the urinary PGE_2 metabolite. Finally, 15-PGDH expression and activity are significantly down-regulated in human colorectal carcinomas relative to matched normal tissue. In summary, these results suggest a novel tumor suppressive role for 15-PGDH due to loss of expression during colorectal tumor progression.

INTRODUCTION

Multistep carcinogenesis unfolds as stochastic mutations and epigenetic changes accumulate within individual cells. In concert with stromal influences, those cells that develop the correct combination of mutations in tumor suppressor genes and oncogenes become fully transformed (1). As an example, the dysregulation of balanced prostaglandin metabolism *in vivo* contributes to carcinogenesis. Numerous reports have demonstrated the increased expression of cyclooxygenase-2 (COX-2)[1] in a variety of human malignancies (2, 3), and higher COX-2 expression correlates with a poor clinical outcome (4). Furthermore, targeted overexpression of COX-2 in mouse mammary tissue leads directly to the development of breast carcinomas in transgenic mice (5). Elevated levels of COX-2-derived prostaglandin E_2 (PGE_2) are associated with resistance to programmed cell death (6) as well as stimulation of cell migration, cell proliferation, and angiogenesis (7). Although steady-state tissue levels of PGE_2 depend on relative rates of biosynthesis and breakdown, virtually all reports examining the role of PGE_2 in physiology and disease have focused solely on the cyclooxygenase-dependent formation of this bioactive lipid. A plausible complementary pathway yielding increased local levels of PGE_2 in cancer involves reduced degradation of PGE_2 by NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH).

15-PGDH catalyzes the rate-limiting step of prostaglandin catabolism (8). The human gene is located on chromosome 4 and encodes a 29-kDa protein that oxidizes the 15(*S*)-hydroxyl group of prostaglandins to yield inactive 15-keto metabolites (9). Genetic deletion of *15-Pgdh*

leads to increased tissue levels of PGE_2 (10). Although previous studies on the distribution and activity of 15-PGDH have focused primarily on parturition and uterine biology, recent data suggest that 15-PGDH plays a role in carcinogenesis (11, 12). Although 15-PGDH may promote certain androgen-sensitive prostate cancers (13), preliminary reports on medullary thyroid and transitional bladder cancers suggest that the loss of 15-PGDH expression contributes to malignancy (14, 15). These conflicting reports are intriguing, and although no mechanism has been demonstrated *in vivo* that explains its down-regulation, a variety of compounds that modulate the expression and activity of 15-PGDH *in vitro* have been reported, including the hypoglycemic thiazolidinediones (16) as well as NSAIDs and PPARγ agonists (17).

To more completely understand prostaglandin function in epithelial biology, our laboratory sought to identify pathways complementary to COX-2 by which PGE_2 might accumulate and contribute to colorectal carcinogenesis. The present study examines the hypothesis that decreased expression of 15-PGDH correlates with colorectal tumor formation. We present data suggesting that 15-PGDH is down-regulated in malignant disease relative to normal tissue and that epidermal growth factor (EGF) and indomethacin can modulate 15-PGDH expression in colorectal carcinoma cells. We also provide functional data both *in vitro* and *in vivo* showing that 15-PGDH expression levels correlate with enzymatic activity and production of the urinary PGE_2 metabolite (PGE-M). Here we report the first indication that decreased catabolism of PGE_2 may regulate tumor formation in the intestine. These data are suggestive of a previously unrecognized pathway in colorectal carcinogenesis, whereby elevated PGE_2 levels derive in part from reduced expression and activity of 15-PGDH. Ultimately, this novel observation may shed light on the adverse clinical outcomes of patients with high levels of tumor-derived cyclooxygenase-2.

EXPERIMENTAL PROCEDURES

Reagents—EGF was purchased from Sigma, and indomethacin, ciglitazone, and GW9662 were purchased from Cayman Chemical (Ann Arbor, MI). Erlotinib (Tarceva™) was obtained from Genentech (San Francisco, CA). FirstChoice mouse and human Northern blots were purchased from Ambion (Austin, TX). The Cancer Profiling Array II was purchased from BD Biosciences. Antibody to Cox-2 (160107) was purchased from Cayman Chemical, and rabbit antiserum against human 15-PGDH was a kind gift from Dr. Hsin-Hsiung Tai (University of Kentucky, Lexington, KY). The Northern blot probe for *Cox-2* was generously provided by Dr. Sanjoy Das (Vanderbilt University, Nashville, TN), and that for *15-Pgdh* was a kind gift from

Dr. Beverly Koller (University of North Carolina, Chapel Hill, NC). The Northern probe for *15-PGDH* was obtained by SpeI digestion of IMAGE clone ID 3638799 from the American Type Culture Collection (Manassas, VA).

Animals and Cell Culture—C57BL/6 and C57BL/6-Apc$^{Min+/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, ME). C57BL/6-*Pgdh*$^{-/-}$ mice were a generous gift from Dr. Beverly Koller. The mice were housed and fed with standard mouse diet in the Animal Care Facility according to National Institutes of Health and institutional guidelines for laboratory animals. LS-174T, HCT-15, HCT-116, DLD-1, HT-29, CaCo-2, Colo 201, LoVo, and SW480 cells were purchased from the American Type Culture Collection, and HCA-7 cells were a generous gift from Dr. Susan Kirkland. LS-174T, HCT-15, HCT-116, HCA-7, and HT-29 cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 atmosphere. SW480, Colo 201, and DLD-1 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 atmosphere. CaCo-2 cells were maintained in minimum essential medium, and LoVo cells were maintained in Ham's F-12K medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 atmosphere.

Northern Blotting—Total cellular RNA was isolated from cells by TRI reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer's protocol. 5 µg of total RNA was fractionated with a MOPS-formaldehyde agarose gel and transferred to Hybond N1 membrane (Amersham Biosciences). Following UV cross-linking, the blots were prehybridized for 30 min at 42 °C in Hybrisol I (Intergen Company, Purchase, NY) and then hybridized using ^{32}P-labeled cDNA in the same buffer at 42 °C, washed, and subjected to autoradiography.

Western Blotting—Cells were washed with phosphate-buffered saline and lysed with radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitors from Roche Applied Science). Protein concentrations were measured using Bio-Rad reagent. Proteins were then separated on precast 4–20% SDS polyacrylamide gels (Invitrogen) and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk in Tris-buffered saline, 0.1% Tween 20 and incubated with primary antibody (15-PGDH, 1/15,000 and β-actin, 1/2,000) overnight at 4 °C. The membranes were then treated with horseradish peroxidase-conjugated secondary antibody and developed using an ECL kit (Amersham Biosciences).

15-PGDH Activity Assay—15-PGDH activity was assayed by measuring the transfer of tritium from 15(*S*)-[15-^{3}H]PGE_2 to glutamate through coupling 15-PGDH and glutamate

dehydrogenase as described previously (18). Briefly, the reaction mixture contained 5 μM NH_4Cl, 1 μM α-ketoglutarate, 1 μM NAD^+, 1 nM 15(*S*)-[15-^{3}H]PGE_2, 100 μg of glutamate dehydrogenase, and crude enzyme extract in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.5. The reaction was allowed to proceed for 10 min at 37 °C and was terminated by the addition of 0.3 ml of 3% aqueous charcoal suspension. Supernatant radioactivity following centrifugation (1000 x *g*, 5 min) was determined by liquid scintillation counting. Calculation of oxidized PGE_2 levels was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(*S*)-hydroxyl group of 15(*S*)-[15-^{3}H]PGE_2.

Liquid Chromatography and Mass Spectrometry—The major urinary metabolite of PGE_2, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), was measured in urine as described previously (19). Briefly, PGE-M contained in 400 μl of mouse urine was derivatized using methoximine HCl (16% w/v) in a 1.5 M sodium acetate solution, diluted in water, and adjusted to pH 3 with acetic acid. Samples were purified by extraction using a C18 SepPak, after which 12.4 ng of *O*-[$^2H_6$]methyloxime PGE-M internal standard was added. Samples were dried under nitrogen, resuspended in 50 μl of mobile phase A (95:4.9:0.1 (v/v/v) 5 mM ammonium acetate:acetonitrile:acetic acid), and analyzed by liquid chromatography tandem mass spectrometry as described previously (19).

Human Colorectal Tissue Samples—Human colorectal tumor specimens were obtained from surgical resections, with Vanderbilt Internal Review Board approval. For each tumor sample, matched adjacent normal mucosa was collected for comparison. All samples were snap-frozen and stored in liquid nitrogen until use. RNA and protein from tissues were processed as described above.

Statistical Analysis—Each experiment was performed at least three times, and data were expressed as the means ± S.E. Statistical significance was determined by paired Student's *t* test. *p* values <0.05 were considered statistically significant.

RESULTS

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Heterogeneous Expression of 15-PGDH with Highest Levels in the Large Intestine—As a preliminary study, we examined a wide range of tissue types to identify the physiologic expression pattern of 15-PGDH. 15-PGDH is known to be ubiquitously expressed in several organs in mammals (8), and total RNA was analyzed by Northern blot for tissues from human (Fig. 1*A*) and mouse (Fig.

1*B*). In humans, we found the highest expression levels in the large intestine (Fig. 1*A*), but elevated *15-PGDH* mRNA was also detected in the lung, the liver, and the small intestine (Fig. 1, *A* and *B*). The lungs and liver are known to play an important role in prostaglandin metabolism *in vivo* (20), but this is the first report suggesting that 15-PGDH plays a role in the intestine.

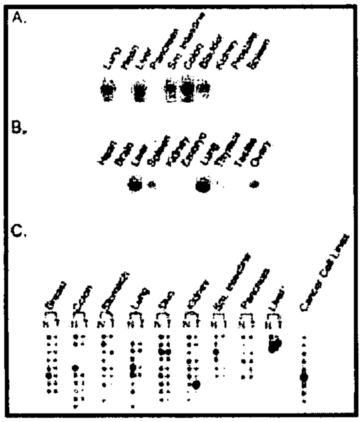

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FIG. 1.
15-PGDH expression in normal and pathologic tissues. FirstBlot membranes blotted with RNA from normal human (*A*) and mouse (*B*) tissues were probed for expression of *15-PGDH*. Each blot contains 2 µg of poly(A) RNA/lane. *C*, a Cancer Profiling Array II was hybridized with a *15-PGDH* cDNA probe and examined for differential expression in paired normal (*N*) and tumor (*T*) samples from the following tissues: breast (*n* = 10), colon (*n* = 10), stomach (*n* = 10), lung (*n* = 10), skin (*n* = 10), kidney (*n* = 9), small intestine (*n* = 7), pancreas (*n* = 7), and liver (*n* = 3). Nine cancer cell lines were included as follows (from *top* to *bottom*): HeLa (cervical carcinoma), Daudi (Burkitt's lymphoma), K562 (CML), HL60 (promyelocytic leukemia), G361 (melanoma), A549 (lung carcinoma), MOLT4 (ALL), SW480 (colorectal carcinoma), and Raji (Burkitt's lymphoma).

These findings prompted us to determine whether the relative expression of 15-PGDH is altered in normal *versus* malignant tissues. A preliminary analysis of matched normal/tumor samples from multiple human tumors revealed that 15-PGDH was dysregulated in a wide range of human cancers, including those of the breast, stomach, lung, skin, kidney, small intestine, pancreas, liver, and colon (Fig. 1*C*). We chose to focus our attention initially on the role of 15-PGDH in colorectal cancer because of our long term focus on understanding prostaglandin function in intestinal biology.

Down-regulation of 15-PGDH Expression in Colorectal Carcinoma Cells—To assess the expression pattern and modulation of 15-PGDH in cultured colorectal carcinoma cells, we evaluated the following cell lines: HCT-15, HCT-116, HCA-7, HT-29, DLD-1, SW480, and LS-174T (Fig. 2*A*). Examination of the additional colorectal carcinoma cell lines CaCo-2, Colo 201, and LoVo revealed that only LoVo cells expressed *15-PGDH* (data not shown). Most colorectal carcinoma cells (7 of 10) examined in this study displayed little or no *15-PGDH* mRNA, although certain cell lines retained varying levels of 15-PGDH expression, including HCT-15 and HCA-7. These results are consistent with our *in vivo* findings that most colorectal carcinomas have significant reductions in 15-PGDH levels (see Fig. 4).

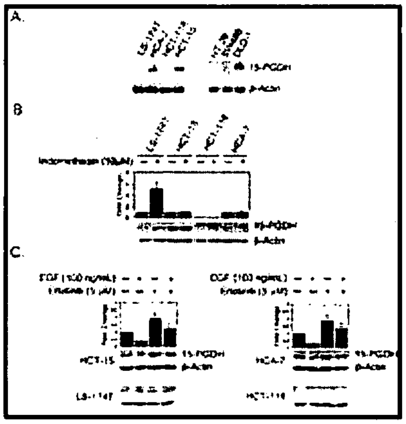

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FIG. 2.
Expression and modulation of 15-PGDH in multiple colorectal cell lines. *A*, total RNA was isolated following harvest of several colorectal carcinoma cell lines. 5 µg of RNA were loaded, and the levels of *15-PGDH* mRNA were determined by Northern blot analysis. *B*, colorectal carcinoma cells were grown for 24 h (HCT-15, HCT-116, and HCA-7) and 48 h (LS-174T) in serum-free media prior to the addition of indomethacin (10 µM) for 72 h. Following the isolation of total cellular protein, 30 µg of protein were separated by SDS-PAGE and visualized with 15-PGDH-specific antibody and β-actin. Data from three independent experiments were quantified by densitometry for the LS-174T, HCT-15, and HCA-7 cell lines and are shown as the means ± S.E. Indomethacin-treated samples were normalized to untreated controls. *C*, colorectal carcinoma cells were grown for 24 h (HCT-15, HCT-116, and HCA-7) and 48 h (LS-174T) in serum-free media prior to the addition of EGF (100 ng/ml) for 24 h with or without 1 h of pretreatment with 5 µM erlotinib. Following the isolation of total cellular protein, 30 µg of protein were separated by SDS-PAGE and visualized with 15-PGDH-specific antibody and β-actin. Data from three independent experiments were quantified by densitometry for both the HCT-15 and HCA-7 cell lines and are shown as the means ± S.E. EGF- and erlotinib-treated samples were normalized to untreated control samples.

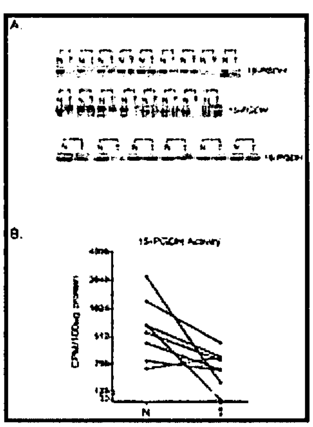

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FIG. 4.
Loss of 15-PGDH expression and activity in human colorectal cancer tissues. *A*, total RNA and protein were isolated from 23 individual human colorectal cancer tissues and matched normal mucosa. Equal amounts of RNA and protein were analyzed for 15-PGDH expression. *N*, normal mucosa; *T*, tumor tissue. *B*, determination of 15-PGDH activity is shown in eight human colorectal cancer tissues and matched normal mucosa.

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Four representative lines were employed in the following experiments: HCT-15, HCT-116, HCA-7, and LS-174T. This panel of cells was selected to span a full range of colorectal carcinoma lines with regard to 15-PGDH expression and endogenous prostaglandin levels. HCT-15 and HCT-116 cells do not express appreciable amounts of cyclooxygenase and have high (HCT-15) and low (HCT-116) endogenous levels of 15-PGDH expression, respectively. LS-174T cells express low levels of 15-PGDH and cyclooxygenase but have been shown to be extremely sensitive to exogenous PGE_2 treatment (21). Finally, HCA-7 cells have high levels of COX-2 and endogenous prostaglandins as well as moderate levels of 15-PGDH.

To study the regulation of 15-PGDH expression *in vitro*, we focused initially on indomethacin and ciglitazone, compounds reported to modulate 15-PGDH in other experimental models. Indomethacin is a widely used NSAID with a number of pharmacologic activities beyond the inhibition of COX-1 and COX-2 activity. Previous reports indicate that indomethacin induces 15-PGDH expression in certain contexts (14, 22, 23), and our results suggested that 10 µM indomethacin could induce 15-PGDH expression in LS-174T cells (p = 0.035) yet have no apparent effect in HCA-7, HCT-15, and HCT-116 cells (Fig. 2*B*). Quantitation of 15-PGDH data by densitometry supports these findings.

While evaluating additional mediators that can modulate the expression of 15-PGDH, we examined the PPARγ agonist ciglitazone with and without pretreatment with an irreversible PPARγ-specific antagonist, GW9662. Although previous reports indicate that ciglitazone is a potent antagonist of 15-PGDH enzymatic activity with an IC_{50} of 2.7 µM (17), we did not see a significant effect of this PPARγ ligand on 15-PGDH expression in our studies (data not shown). To evaluate whether 15-PGDH may serve as a tumor suppressor gene, we sought to identify known oncogenic pathways that could alter expression of 15-PGDH.

EGF is an established mediator of proliferation in a variety of human cancers. In addition, signal transduction pathways downstream from the EP4 PGE_2 receptor have been shown to transactivate the EGF receptor (EGFR) (24, 25). We sought to identify whether stimulation or blockade of the EGFR (ErbB1) could modulate expression of 15-PGDH. Consistent with a tumor-suppressor role for 15-PGDH, EGF down-regulated 15-PGDH protein in HCT-15 (p = 0.013) and HCA-7 (p = 0.008) cells relative to untreated controls. Conversely, the inhibition of this pathway with the EGFR-specific tyrosine kinase inhibitor (erlotinib) led to the increased expression of 15-PGDH in both HCT-15 (p = 0.016) and HCA-7 (p = 0.070) (Fig. 2*C*). Pretreatment with erlotinib for 1 h prior to the addition of EGF largely blocked the down-regulation of 15-PGDH compared with EGF treatment alone (Fig. 2*C*). To our knowledge, this is

the first observation that stimulation of the EGFR signaling cascade can down-regulate prostaglandin catabolism.

Modulation of 15-PGDH Expression and Activity in Vivo—Coordinate regulation of increased PGE_2 production and inhibition of PGE_2 degradation may increase local tissue levels of PGE_2. The relevance of this dysregulated state to carcinogenesis *in vivo* is emphasized by our recent study indicating that treatment of $Apc^{Min+/-}$ mice with PGE_2 significantly accelerates adenoma growth (26). Thus, we next evaluated *15-Pgdh* levels in $Apc^{Min+/-}$ adenomas to see whether decreased catabolism could provide a partial explanation for the stimulatory effect of exogenous PGE_2 on adenoma growth *in vivo*. Northern and Western analysis of C57BL/6 and C57BL/6-$Apc^{Min+/-}$ mouse intestine showed that wild-type C57BL/6 mice exhibited strong expression of *15-Pgdh* mRNA (Fig. 3*A*). The situation was reversed in adenomas of 15-week-old $Apc^{Min+/-}$ mice, in which the expression of *15-Pgdh* was markedly reduced (Fig. 3*A*). Interestingly, intestinal mucosa with a microscopically normal appearance maintained the expression of *15-Pgdh* in these mice. By contrast, both Cox-2 mRNA and protein levels were low in the normal mucosa but significantly increased within the polyp microenvironment (Fig. 3, *A* and *B*). This finding is very interesting in light of the recent report that basal EGFR activity is increased in $Apc^{Min+/-}$ intestinal adenomas (27), combined with our current observation that the activation of EGFR lowers 15-PGDH expression.

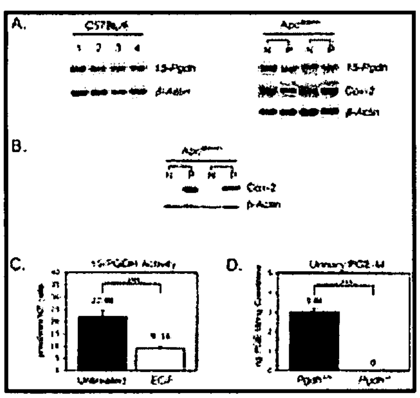

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FIG. 3.
Loss of 15-PGDH decreases PGE_2 catabolism and contributes to carcinogenesis *in vivo*. *A*, Northern analysis of *15-Pgdh* and *Cox-2* expression in the small intestine of control C57BL/6 and $Apc^{Min+/-}$ mice at 15 weeks of age. *N*, normal mucosa; *P*, polyp tissue. *B*, Western analysis of *Cox-2* expression in the small intestine of $Apc^{Min+/-}$ mice at 15 weeks of age. *C*, determination of 15-PGDH activity in HCT-15 cells treated with and without EGF (100 ng/ml) for 24 h (n = 6). ***, $p < 0.001$. *D*, comparative urinalysis of pooled $Pgdh^{+/+}$ and $Pgdh^{-/-}$ mice for the PGE_2 metabolite PGE-M (n = 3). ***, $p < 0.001$.

Functional Analysis of 15-PGDH in Vitro and in Vivo—The modulation of 15-PGDH expression by EGF naturally raised questions regarding the functional significance of these changes. To assess whether 15-PGDH protein levels correlate with enzymatic catabolism of PGE_2, 15-PGDH activity was assayed *in vitro* as described previously (18). The treatment of HCT-15 cells with

EGF (100 ng/ml) for 24 h decreased 15-PGDH enzymatic activity by 59% relative to untreated control cells ($p < 0.001$) (Fig. 3*C*). This reduction mirrors the decrease in protein levels under these conditions and provides evidence that protein expression and enzymatic activity of 15-PGDH correlate *in vitro*.

Recent reports indicate that genetic disruption of *15-Pgdh* in the mouse results in increased tissue levels of PGE_2 (10). To determine how the loss of 15-PGDH expression affects PGE_2 catabolism *in vivo*, we measured PGE-M, the major urinary metabolite of PGE_2 (19). Interestingly, analysis of urine collected from wild-type C57BL/6 and C57BL/6-*Pgdh*$^{-/-}$ mice showed that PGE-M is absent in urine collected from *Pgdh*$^{-/-}$ mice compared with control animals ($p < 0.001$) (Fig. 3*D*). This provides strong evidence for the hypothesis that loss of 15-PGDH expression alone can directly disrupt catabolism of PGE_2 and contribute to elevated levels of bioactive PGE_2.

Loss of 15-PGDH Expression and Activity in Human Colorectal Cancers—Finally, to extend our *in vivo* studies and assess the biological relevance of these observations, we examined clinical samples of human colorectal carcinomas. A comparison of human colon cancers and matched normal tissue revealed greatly reduced expression of 15-PGDH in malignant tissue relative to normal colonic mucosa. Northern and Western blotting indicated that 15-PGDH levels were decreased in 85% of the 23 pairs of colon carcinoma samples compared with adjacent normal mucosa (Fig. 4*A*). To assess the functional significance of these findings, 15-PGDH enzymatic activity was compared in eight paired human colorectal cancer tissues and matched normal mucosa. 15-PGDH activity was found to reflect changes in expression levels ($p = 0.019$) (Fig. 4*B*). These data support the hypothesis that 15-PGDH expression and activity are down-regulated in colorectal cancer.

DISCUSSION

PGE_2 levels are elevated significantly at sites of inflammation and malignancy; these findings are often attributed to the increased expression of the inducible cyclooxygenase isoenzyme COX-2. The well studied functions of COX-2-derived PGE_2 in malignant and metastatic disease indicate a role in modulation of apoptosis, stimulation of angiogenesis, and promotion of tumor invasion (6, 7). A large body of evidence has revealed a 40–50% reduction in risk and mortality from colorectal cancer in individuals taking NSAIDs regularly, either in the context of sporadic colorectal cancers or in familial adenomatous polyposis. Both conditions are

associated with high levels of PGE_2, and the protective effects of NSAIDs are due, at least in part, to inhibition of cyclooxygenase-dependent formation of PGE_2 (28–30).

While virtually all studies have focused on pathologic overproduction by COX-2, steady-state levels of PGE_2 depend on the relative rates of biosynthesis and breakdown. Reduced degradation of PGE_2 by the rate-limiting catabolic enzyme 15-PGDH provides a potential alternative mechanism for the local accumulation of PGE_2. The role of 15-PGDH in malignancy has not been addressed adequately. To increase our understanding of prostaglandin function in carcinogenesis, the present study sought to test the hypothesis that dysregulation of 15-PGDH is associated with colorectal tumorigenesis. We employ a variety of approaches to support our contention that 15-PGDH is down-regulated in colorectal cancer both *in vitro* and *in vivo*. Several human colorectal carcinoma cell lines, $Apc^{Min+/-}$ mice, and human sporadic colorectal cancer samples show decreased 15-PGDH expression and activity relative to matched normal tissues.

These findings naturally raise questions regarding the regulation of 15-PGDH expression at the molecular level. Rao *et al.* (11) report that inhibition of 15-PGDH activity by the isoflavonoid genistein increases tumor burden in azoxymethane-treated rats. Although previous studies show induction of 15-PGDH by indomethacin in certain contexts (14, 22, 23), we only observed induction of 15-PGDH by indomethacin in one of four colorectal carcinoma cell lines (LS-174T). Examination of additional cell lines and further testing *in vivo* are necessary to elucidate whether indomethacin regulates 15-PGDH in the intestine. Conversely, the inhibition of 15-PGDH expression and activity by EGF suggests a previously unknown mechanism by which EGFR ligands positively regulate local PGE_2 levels. While ErbB2 is known to induce COX-2 (31), the current study suggests that EGF can decrease the expression of 15-PGDH in colonocytes. Our findings with EGF in HCT-15 and HCA-7 are complemented by data showing induction of 15-PGDH following inhibition of EGFR tyrosine kinase activity with erlotinib. Although the majority of EGFR studies focus on activation of intracellular signaling cascades, recent data from Lu *et al.* (32) support the view that EGF-induced negative regulation of gene function can promote tumor formation.

The loss of 15-PGDH expression in colorectal carcinomas is quite intriguing in light of previous reports indicating that up-regulation of EGFR occurs in 60–80% of colorectal cancers (33–35). The expression of EGFR is known to be associated with poor survival in patients with colorectal cancer (36), and recent studies indicate that inhibitors of EGFR signaling have clinically significant activity when given to patients with colorectal cancer who are refractory to other treatment (37). As several anticancer drugs that target EGFR signaling are currently being developed, our present results may help to explain the clinical efficacy of these new agents.

These data may also be quite significant when coupled with our original report (38) that COX-2 is an EGF/transforming growth factor-α-inducible gene in intestinal epithelial cells, with robust enhancement of prostaglandin production following EGFR activation. Other reports (25) from our group support a feed-forward mechanism whereby COX-2-derived PGE_2 can transactivate the EGFR. The present study suggests that 15-PGDH is negatively regulated downstream from activated EGFR, allowing PGE_2 to accumulate and activate this cycle repeatedly. AP-1, Ets, and cAMP-responsive element-binding (CREB) proteins have been implicated in the transcriptional regulation of 15-PGDH (39). Future studies will determine whether any of these factors mediate EGF-dependent suppression of 15-PGDH transcription in the intestine.

Data presented in this paper indicate that the regulation of local PGE_2 levels in human colorectal cancers is complex, involving both increased expression of COX-2 and loss of 15-PGDH. Although 15-PGDH is known to play an important role in physiologic processes such as parturition through modulation of local prostaglandin levels, our data show that 15-PGDH may inhibit cellular transformation by metabolizing local PGE_2 produced at the tumor site. Coordinate regulation of increased PGE_2 production and inhibition of PGE_2 degradation lead to elevated levels of PGE_2, and the relevance of this dysregulated state to carcinogenesis *in vivo* is emphasized by our recent study (26) indicating that treatment of $Apc^{Min+/-}$ mice with PGE_2 significantly accelerates adenoma growth.

$Apc^{Min+/-}$ mice develop intestinal adenomas spontaneously and have elevated levels of PGE_2 at 15 weeks that correlate closely with multiplicity of intestinal polyps (40). Previously attributed solely to elevated COX-2 activity, our data in 15-week-old $Apc^{Min+/-}$ mice suggest that loss of 15-Pgdh also correlates with tumor progression in this setting. Given that the loss of 15-Pgdh decreases PGE_2 catabolism, our data also support the hypothesis that 15-Pgdh serves an important homeostatic function involving degradation of PGE_2. Loss of enzyme expression specifically within the tumor microenvironment coincides with adenoma formation in 15-week-old $Apc^{Min+/-}$mice, a process that can be accelerated by treatment with exogenous PGE_2 (26).

In summary, this study examines 15-PGDH expression in both normal and tumor tissues of the mouse and human intestine, with preliminary analysis of 15-PGDH regulation *in vitro*. We show that 15-PGDH mRNA and protein levels are significantly reduced in human colorectal cancers relative to normal mucosa. A similar decrease in 15-PGDH expression is found specifically within the tumor microenvironment of intestinal adenomas in 15-week-old $Apc^{Min+/-}$ mice. Loss of 15-PGDH expression correlates with reduced enzymatic activity *in vitro* and *in vivo*. We

also show that the loss of 15-PGDH function *in vivo* markedly decreases metabolic inactivation of PGE_2. Taken together, our data provide the first evidence that reduced catabolism of PGE_2 may promote colorectal tumorigenesis. These preliminary findings provide a novel framework for further investigation into the regulation of 15-PGDH expression and function *in vivo*.

FOOTNOTES

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[1] The abbreviations used are: COX, cyclooxygenase; PG, prostaglandin; PGDH, PG dehydrogenase; NSAID, nonsteroidal anti-inflammatory drug; PPAR, peroxisome proliferator-activated receptor; EGF, epidermal growth factor; EGFR, EGF receptor; PGE-M, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid; MOPS, 4-morpholinepropanesulfonic acid.

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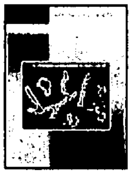

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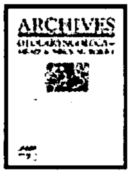

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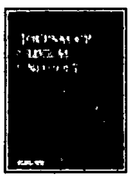

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